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Article 34

PCT/US 98/15387
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CLAIMS

What is claimed is:

sub a'

1. A method of producing nuclear transfer embryos from donor cells of one species and recipient oocytes from another species comprising:

inducing the donor cells to undergo G_0 arrest;

fusing said donor cell to an enucleated recipient oocyte of another species to create a nuclear transfer embryo;

and activating said nuclear transfer embryo.

2. The method of claim 1 wherein said G_0 arrest of donor cells is induced by culture in low serum medium.

3. The method of claim 1 wherein said donor cells are selected from the group consisting of embryonic derived cells, germ cells, somatic cells, and genetically modified cells.

4. The method of claim 1 wherein said enucleated recipient oocyte is an enucleated bovine recipient oocyte.

sub a²

5. The method of claim 4 wherein said enucleated bovine recipient oocyte is selected from the group of bovine oocytes undergoing nuclear maturation within 16 hours of beginning in vitro culture.

6. The method of claim 1 wherein said enucleated bovine recipient oocyte and said donor cell are fused by electric pulse to form a nuclear transfer embryo.

7. The method of claim 1 wherein said fusion is performed 16-32 hours after the beginning of in vitro culture.

sub a³

8. The method of claim 1 wherein said nuclear transfer embryo is activated by elevating intracellular calcium and the incubating with a serine threonine kinase inhibitor.

AMENDED SHEET

9. The method of claim 8 wherein intracellular calcium is elevated by incubation with ionomycin and the serine threonine kinase inhibitor is DMAP.

10. The method of claim 1 wherein said activation is 16-32 hours after the beginning of in vitro culture.

11. The method of claim 6 wherein said fusion is 16-52 hours after the beginning of in vitro culture.

sub Q4

12. An embryo produced by the method of claim 1.

13. A method of producing nuclear transfer embryos from a donor cell of one species and a bovine recipient oocyte comprising:

culturing non-bovine donor cells selected from the group consisting of embryonic derived cells, somatic cells, germ cells, and genetically modified cells in low serum medium so that said donor cells are induced to arrest in the G_0 stage of the cell cycle;

selecting a bovine recipient oocyte which has completed nuclear maturation before 16 hours from the beginning of in vitro culture;

enucleating said bovine recipient oocyte after 16-32 hours of in vitro culture;

placing said donor cell under the zone pellucida of said enucleated oocyte so that said donor cell contacts said enucleated oocyte;

fusing said donor cell with said enucleated oocyte by electric pulse at 16-32 hours after the beginning of in vitro culture to create a nuclear transfer embryo;

and activating said nuclear transfer embryo by sequential incubation with ionomycin and 6-dimethylaminopurine at 16 to 32 hours after the beginning of in vitro culture.

14. The embryo produced by the process of claim 13.

15. A nucl ar transfer embryo comprising cytoplasm and cell membrane from one species and differentiated cytoplasm, differentiated cell membrane, and nuclei derived from a differentiated cell of another species.